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Katinger et al.

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(54) **HIV-VACCINES**

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(22) Filed: Jul. 30, 1998

Related U.S. Application Data

(60) Division of application No. 08/478,536, filed on Jun. 7, 1995, now Pat. No. 5,911,989, which is a continuation-in-part of application No. PCT/EP95/01481, filed on Apr. 19, 1995.

(51) Int. Cl.⁷ C07K 16/00; A61K 39/00; A61K 39/21; C12Q 1/70; G01N 33/53

(52) U.S. Cl. 530/388.35; 424/192.1; 424/208.1; 435/5; 435/7.1; 435/339.1

(58) Field of Search 424/192.1, 208.1; 530/388.35; 435/5, 7.1, 339.1

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(57) ABSTRACT

Disclosed are antibodies which can be used for the manufacture of vaccines for active and/or passive immunization of persons in need of such treatment. The invention also provides for human monoclonal antibodies that are functionally equivalent to the above-mentioned antibodies produced by any one of the cell lines CL1 through CL6 (deposited at the European Collection of Animal Cell Cultures (ECACC) at the PHLS in Porton Down, Salisbury, UK). Also provided are hybridoma and/or CHO cell lines producing any one of the antibodies disclosed and claimed herein. Also provided are mixtures of antibodies of the present invention, as well as methods of using individual antibodies or mixtures thereof for the detection, prevention and/or therapeutic treatment of HIV-1 infections in vitro and in vivo.

8 Claims, 6 Drawing Sheets

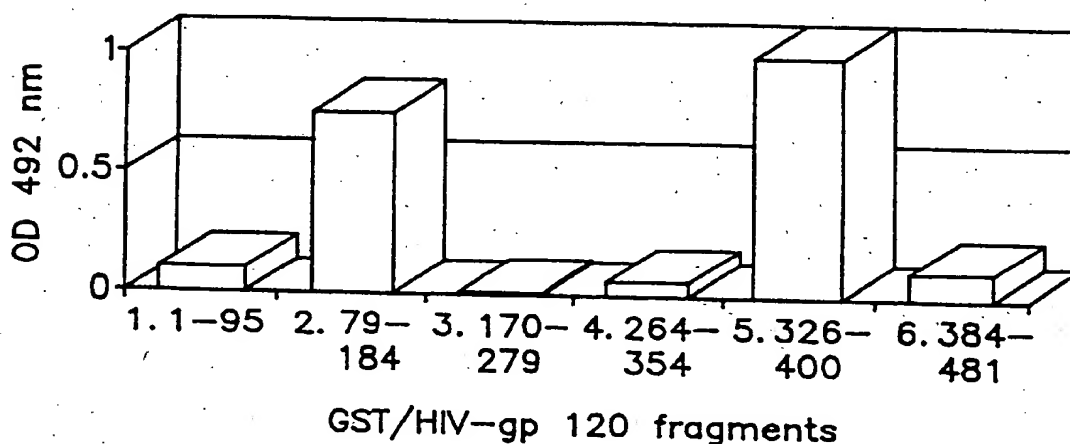


EXHIBIT B

FIG. 1

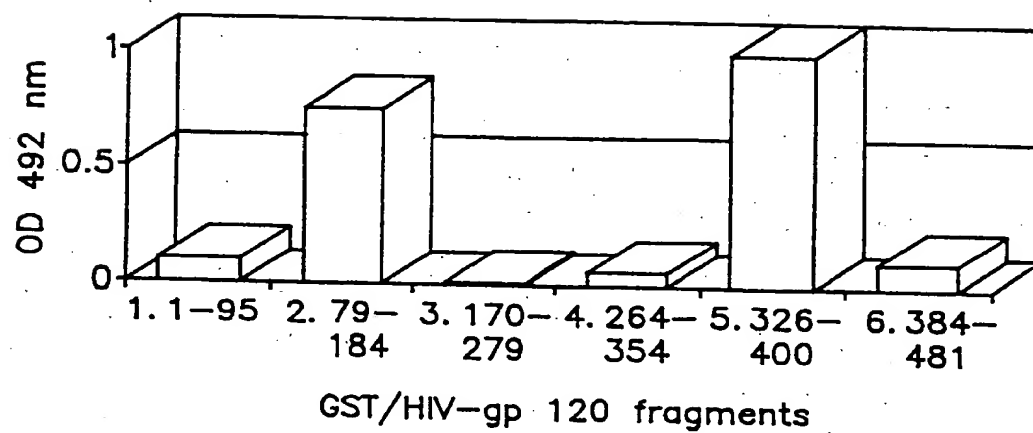


FIG. 2

A**1 2 3**

—

B**1 2 3**

—

—

—

—142—
—97—
—50—
—35—
—30—

FIG. 3

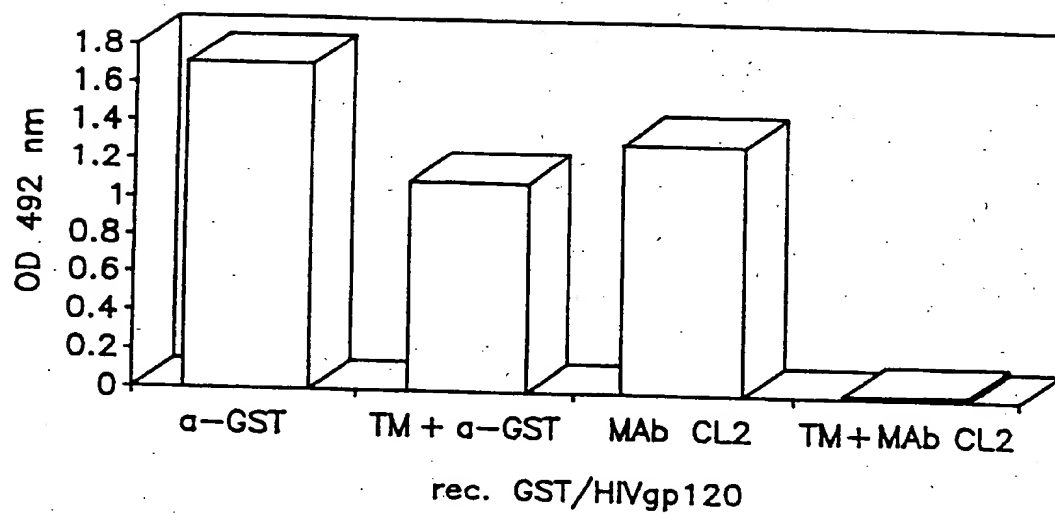


FIG. 4A

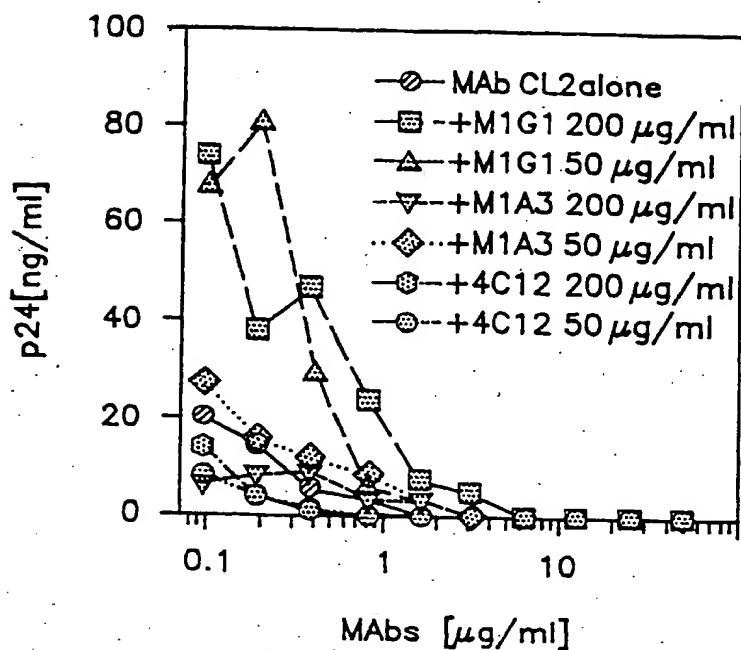


FIG. 4B

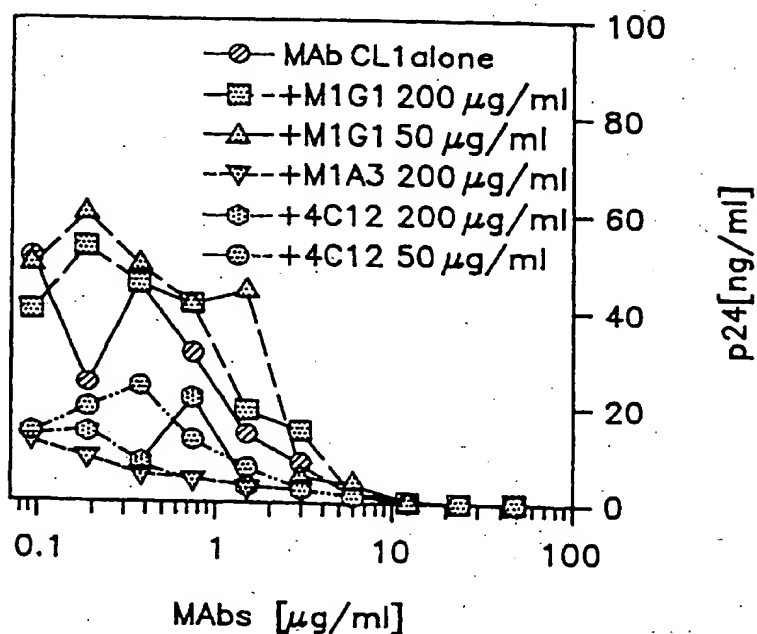


FIG. 5A

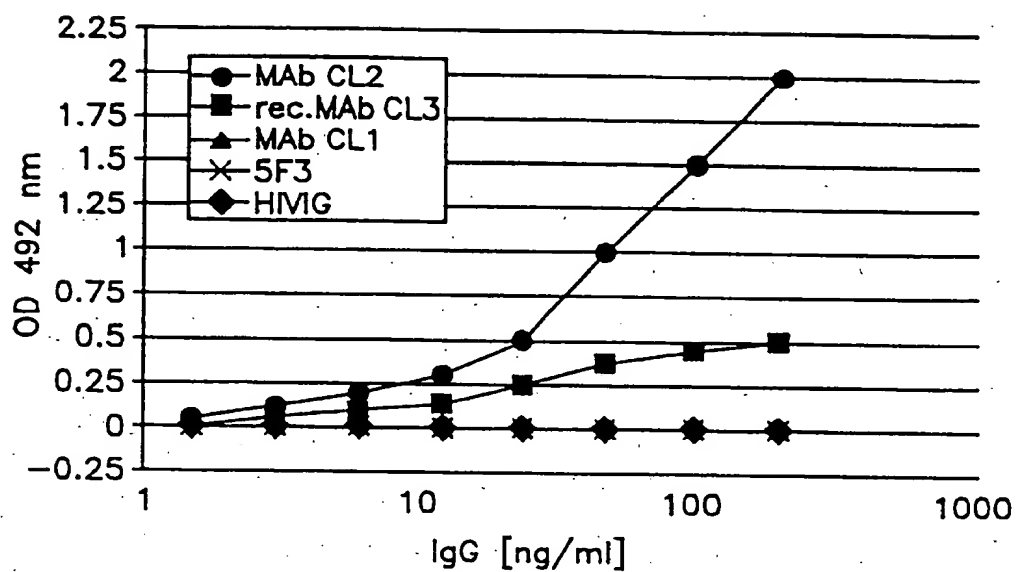


FIG. 5B

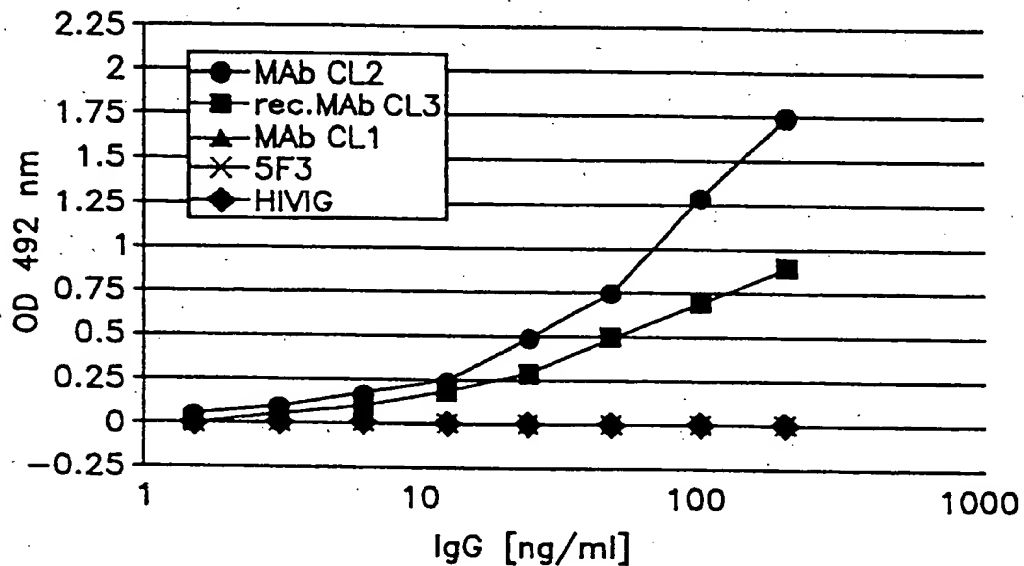
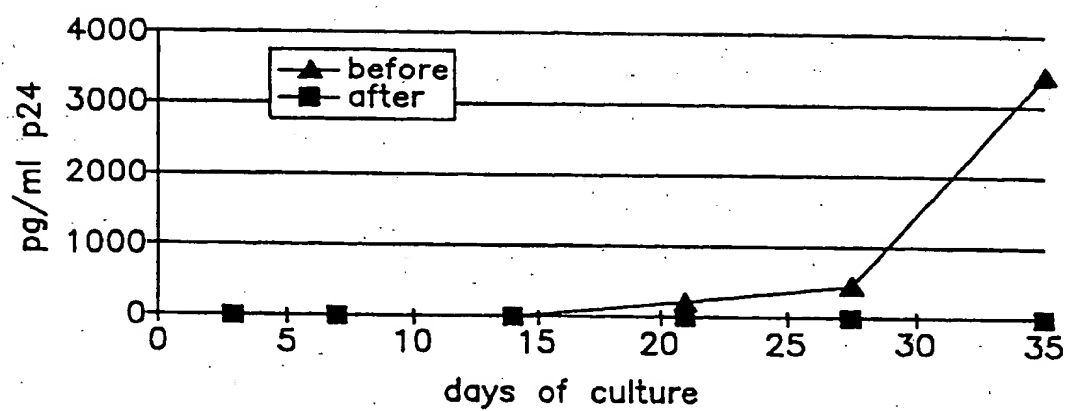


FIG. 6



HIV-VACCINES

This application is a divisional of U.S. application Ser. No. 08/478,536 filed on Jun. 7, 1995 which issued as U.S. Pat. No. 5,911,989, which is a Continuation-in-Part Application of PCT International Application No. PCT/EP95/01481 filed on Apr. 19, 1995 under 35 U.S.C. § 371, the entire contents of each of the above identified applications are herein incorporated by reference.

BACKGROUND OF THE INVENTION

1. Technical field

The present invention is in the field of immunology, especially detection, prevention and treatment of HIV-1 infection and AIDS therapy. More particularly, it concerns monoclonal antibodies, drugs and vaccines made from these antibodies and methods based on the use of these antibodies, drugs and vaccines for analytical and/or clinical applications.

2. Description of Related Art

In the sera of human immunodeficiency virus type 1 (HIV-1) infected patients, anti-virus antibodies can be detected over a certain period after infection without any clinical manifestations of the acquired immunodeficiency syndrome (AIDS). At this state of active immune response, high numbers of antigen-specific B-cells are expected in the circulation. These B-cells are used as fusion partners for the generation of human monoclonal anti-HIV antibodies.

Monoclonal antibodies can be produced by known procedures, e.g., as described by R. Kennet et al. in "Monoclonal Antibodies and Functional Cell Lines; Progress and Applications". Plenum Press (New York), 1984.

Further materials and methods applied are based on known procedures, e.g., such as described in J. Virol. 67:6642-6647, 1993.

Monoclonal antibodies and in particular human monoclonal antibodies have been widely used in the last few years in order to improve the understanding of HIV-1 neutralization by antibodies released upon immunization with HIV-1 derived immunogens or upon infection in afflicted patients (J. Virol. 62:2107-2114, 1988; Immunology 76:515-534, 1992; J. Virol. 67:6642-6647, 1993; U.S. Pat. No. 5,087,557). Many efforts have been made to overcome the detrimental capability of the HIV-1 virus to rapidly change its morphology under immunological pressure and thereby to escape the capture by antibodies released from a patient's immune systems or developed and applied by researchers. As a result thereof, there is presently no reliable antibody-leaved (nor any other) vaccine for active or passive immunization on the market. One significant step forward has been made when an antigens determinant on the smaller subunit gp41 of the HIV-1 envelope glycoprotein gp160 was found (EP 570 357 A2), which corresponds to the amino acid sequence "ELDKWA" (SEQ ID NO:11) located at amino acid position number 662 to 667 of gp41 of HIV-1 isolate BH10. The authors report therein an HIV-1 neutralizing human monoclonal antibody specifically binding to said antigenic determinant. The antibody proved to be a powerful tool for biochemical analysis of the binding epitope and its variability. The discovery of the highly conserved state of said gp41-epitope gave rise to the hope of possibly finding a vaccine composition suitable for more reliable prevention of human individuals from HIV-1 infection and/or for more successful therapeutic treatment of infected patients.

The results reported in EP 570 357 A2 motivated the present inventors to intensify their research activities which finally led them to the novel and inventive findings herein disclosed.

However, in spite of promising results of the art relating to the use of HIV-1 neutralizing monoclonal antibodies, there is at least one major drawback to this sort of approach. It lies in the wide-spread use of laboratory strains of HIV-1 isolates, which have become adapted to lab-conditions and are more or less attenuated and hence only poorly—if at all—representative of the properties and behaviour of primary HIV-1 isolates. Consequently, promising vaccine compositions drawn against laboratory HIV-1 strains frequently proved non-efficacious when applied against primary HIV-1 isolates, e.g., of blood samples of infected persons (see J. Cohen, Science 262:980-981, 1993).

The second major drawback was and still is the ability of the HIV-1 virus to escape antibody capture by morphological variation, which very often renders the remarkable efforts of the researchers almost useless. Such escape mutants may be characterized by a change of only one or several of the amino acids within one of the targeted antigenic determinants and may occur, e.g., as a result of spontaneous or induced mutation.

SUMMARY OF THE INVENTION

The present invention therefore provides antibodies which have been found to overcome the disadvantages of the prior art and which can be used for the manufacture of vaccines for active and/or passive immunization of persons in need of such treatment. Such beneficial antibodies are, for instance, produced by any one of the cell lines CL1 through CL6 listed below. The invention also provides for human monoclonal antibodies that are functionally equivalent to the antibodies of CL1 through CL6. These functionally equivalent antibodies substantially share at least one major functional property with an antibody of CL1 to CL6 as herein described, comprising: binding specificity to gp160; binding dependence on glycosylation; reactivity in the presence of tunicamycin; inhibition of infections of human lymphocytes by primary HIV-1 isolates; reactivity towards anti-idiotypes; competition for same binding site; reduction of the HIV-1 level in blood serum after intravenous administration to an infected patient; and/or specific binding to HIV-1 neutralizing antibodies.

It is also an object of the present invention to provide for the hybridoma and/or CHO cell lines producing any one of the antibodies disclosed and claimed herein.

The invention is further directed to mixtures of antibodies according to the present invention, as well as to methods of using individual antibodies or mixtures thereof for the prevention and/or therapeutic treatment of HIV-1 infections in vitro and in vivo, and/or for improved detection of HIV-1 infections.

The cell lines CL1 to CL4 produce monoclonal antibodies recognizing HIV-envelope glycoproteins, and in particular specific antigenic determinants of gp160. The antibodies of CL1 and CL4 recognize and bind to an amino acid sequence of gp41/gp160 corresponding to the epitope located at amino acid position number 662 to 667 ("ELDKWA") of gp41 of HIV-1 isolate BH10 (GenBank accession M15654; (SEQ ID NOS:1-10) numbering as described in the Swissprot database entry ENVSHIV10). The monoclonal antibodies of CL2 and CL3 bind to two different antigenic determinants, more particularly to fragments of gp120/gp160 corresponding to the epitope sequences located at amino acid positions 79 to 184 and 326 to 400 respectively, of processed gp120 of HIV-1 isolate BH10 (GenBank accession M15654; numbering as described in the Swissprot database entry ENVSHIV10).

While the idiotypic antibodies produced by CL1 to CL4 are directed to the capture and neutralization of HIV-1 viruses in vitro and in vivo, the antiidiotypic antibodies released from CL5 and CL6 take an opposite role, i.e., they mimic the viruses, more particularly they mimic the corresponding antigenic determinant(s) of the HIV-1 viruses. The anti-idiotypic antibodies of CL5 and CL6 are of a nature such that they bind to the idiotypic antibody of CL2 at essentially the same location(s) (antigenic determinants) on gp160 as does the virus itself.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the specific binding of a human monoclonal antibody released by hybridoma cell line CL2 with two distinct fragments of glycoprotein subunit gp120 of HIV-1; the numerals indicate the position numbers of the amino acids of the gp120 fragments as herein described;

FIG. 2A shows the failure of a CL2 antibody to bind to the deglycosylated form (lane 3) of gp160 of HIV-1, while MAb CL1 (FIG. 2B) was used as a control because it successfully binds to the deglycosylated gp160;

FIG. 3 shows the reactivity of anti-GST antiserum and a CL2 antibody with a recombinant gp120 in the presence and absence of tunicamycin, while a-GST binds to rec.GST/HIVgp120 in the presence of tunicamycin (at a reduced level) the CL2 antibody does not (bar 4);

FIG. 4A shows the blocking effect of antibodies M1G1 through 4C12 toward HIV-1 neutralizing monoclonal antibodies of the CL2 (plot A) and CL1 (FIG. 4B) cell line in a p24 antigen ELISA; the anti-idiotypic character of M1G1 towards MAb CL2 is demonstrated;

FIG. 5A shows the uniqueness of antibodies of the CL2 and the CL3 (=recomb. CL2) cell lines among a multitude of different anti-HIV antibodies; only the antibodies of CL2 and CL3 are recognized and bound by the antiidiotypic antibodies M1G1 (plot A) and M1A3 FIG. 5B;

FIG. 6 shows HIV-1 neutralizing efficacy of antibodies of cell line CL1 after in vivo application to a human patient; triangles show the HIV-induced formation of syncytia in the serum taken from an infected patient before administration of CL1 antibodies, while squares demonstrate complete failure of syncytia formation after administration of CL1 antibodies.

DETAILED DESCRIPTION OF THE INVENTION

The contents of each of the references cited herein are herein incorporated by reference in their entirety.

When conducting experiments to find novel anti-HIV-1 antibodies the inventors found human monoclonal antibodies which could be shown to efficiently neutralize HIV-1 in vitro including a variety of primary HIV-1 isolates, such as, e.g., primary HIV-1 isolates 92 RW009, 92RW021, 92UG037, 92TH014, 92BR030, N70-2, DJ259 (all obtained from WHO network for HIV-1 isolation and characterization), or WYG, WRB, WSC, WHM (isolated from Austrian patients).

Surprisingly, it turned out that these antibodies recognize and bind to two different antigenic determinants of the glycoprotein gp160 of HIV-1.

Moreover, it appears that the binding target of these antibodies is extraordinarily unique. In a comparative test involving a mixture of 41 different HIV-1 binding antibodies supplied by laboratories from different companies and research institutes, it was shown that no one of the other

antibodies present in the mixture competed with an antibody of the above-identified group, for instance with the human monoclonal antibody from cell line CL2, for binding to the targeted antigenic fragments of gp120/gp160 corresponding to amino acid sequences 79 to 184 and 326 to 400 of processed gp120 of HIV-1 isolate BH10.

Also, investigations of blood serum and blood plasma of HIV-1 infected patients revealed that antibodies of the CL2 type were not present in the samples tested so far. This finding again emphasizes the uniqueness of these HIV-1 neutralizing human monoclonal antibodies and simultaneously indicates that there might exist an extraordinary potential to combat HIV-1 infection, by using these antibodies in a suitable form for the prophylactic and/or therapeutic treatment of human individuals.

Another object of the present invention relates to antibodies of the CL2 type which have been found to bind to the above-mentioned antigenic determinants of gp120/gp160 only if the determinants are present in a glycosylated form; they do not bind to these antigenic glycopeptide fragments when the fragments are deglycosylated, e.g., by the action of Peptide-N-Glycosidase F (EC 3.2.2.18; hereinafter referred to as "PN Gase F").

Still another object of the present invention encompasses human monoclonal antibodies of the CL2 type which are further characterized in that they also specifically bind to a fragment of gp120 produced in the SF9 insect cell/Baculovirus expression system in the absence of tunicamycin, while they do not bind to gp120 fragments expressed in the presence of tunicamycin. Tunicamycin is known for its inhibitory activity toward the glycosylating action of glycosyl transferase in glycoprotein biosynthesis.

Among the antibodies of the CL2 type as disclosed herein, there are also types which inhibit the infection of human lymphocytes by primary HIV-1 isolates such as the ones listed herein, as could successfully be demonstrated by the inventors in in vitro experiments.

The present invention also relates to antibodies of the CL2 type which possess one or more of the above-mentioned properties and which can further be characterized by their special interaction with the anti-idiotypic monoclonal antibodies of hybridoma cell lines CL5 and CL6. While they can be bound by one and/or the other of the two anti-idiotypic antibodies CL5 and CL6, at least part of them is bound by anti-idiotypic huMAb produced by CL6 in a way that results in a specific blockade of the capability of the antibody to inhibit the infection of human lymphocytes by primary HIV-1 isolates.

A further object of the present invention comprises antibodies of the CL2 type which show at least one of the above-mentioned features or properties and which—in addition—have been proved to compete for binding to the antigenic determinants of gp120/160 with the antibody produced by hybridoma cell line CL2. The antibodies of this category are therefore—at least functionally—very closely related with the antibody released by CL2, and can be regarded as functional equivalents to it.

Another object of the present invention is directed to the most beneficial human monoclonal antibody produced by hybridoma cell line CL2. This antibody can be used, e.g. for passive immunization of HIV-1 infected individuals, but may even be more useful as a biochemical tool for developing vaccines applicable in the prevention and/or therapy of HIV-1 infections in vivo.

An attractive object of the present invention comprises the use of recombinant CHO cells for the production of the

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antibodies of the CL2 type. After successful identification of the antigenic determinants recognized and bound by these antibodies, the inventors also succeeded in transforming the respective genetic information into CHO cells, resulting in a stable cell line CL3, which synthesizes the CL2 type antibodies in a more efficient manner than the hybridoma cell line CL2 itself.

In another embodiment, anti-idiotypic antibodies are disclosed which can specifically bind to idiotype antibodies of the CL2 type and/or which can interact with at least some of them in a fashion that eliminates their anti-HIV protective capability, i.e., bars them from inhibiting the infection of human lymphocytes by primary HIV-1 isolates. Such anti-idiotypic antibodies are therefore expected to be conformationally related to the HIV-1 viruses in that they probably contain similar or even identical antigenic fragments of a viral glycoprotein, e.g., of gp160.

The antibodies of the next embodiment seem to be very interesting because they are of an anti-idiotypic type and combine the features of the anti-idiotypic antibodies of the previous embodiment with their ability to induce—upon administration to a mammal, e.g., a human or animal individual—the production and release of anti-HIV-1 antibodies. Optionally, the induced antibodies are of a nature such that they compete for binding to the above specified antigenic determinants of gp120/160 with at least one antibody of the CL2 type as hereinbefore described in any one of the respective embodiments. A special representative of this group of anti-idiotypic antibodies is the one produced by hybridoma cell line CL6.

While the anti-idiotypic antibodies of the preceding embodiment may be used for active immunization of test animals or HIV-1 endangered and preferably not yet infected persons, the antibodies induced upon such active immunization may serve as components of a vaccine for passive immunization or as subjects of investigation to design and/or synthetically or genetically prepare such antibodies. Optionally, these (idiotypic) antibodies are functional equivalents to the CL2 type antibodies, i.e., they compete with the CL2 type antibodies for binding to the above specified antigenic determinants of gp120/gp160.

In a further—most exciting—embodiment of the invention, the human monoclonal antibodies exhibit strong HIV-1 neutralizing activity and bind to the smaller subunit of gp160, hereinafter referred to as gp41/gp160. Preclinical studies have proved that they are able to significantly reduce—upon intravenous administration to a human HIV-1 infected individual—the level of circulating HIV-1 in the blood serum and/or blood plasma of said individual (see Example 8 and FIG. 6).

Moreover, at least part of these antibodies may be further characterized in that they also compete with an idiotype antibody produced by hybridoma cell line CL1 for binding to the gp41/gp160 antigenic determinant. Finally, the antibody produced by said cell line CL1 itself can be regarded as an important member of this group of HIV-1 level reducing antibodies.

Similarly to the situation with the CHO cell line CL3 producing CL2 type antibodies, the inventors also succeeded in cloning a recombinant CHO cell line CL4 producing antibodies which compete with the antibody of CL1 for binding to the gp41/gp160 antigenic determinant and hence may be regarded as more or less close equivalents to the CL1 antibody. Such recombinant CHO cell lines are easier to grow and more efficiently employed in the manufacture of the respective antibodies.

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Various in vitro experiments have proved that the CL2 type antibodies as well as the CL1 type antibodies are able to neutralize a variety of different laboratory and primary HIV-1 isolates including a number of escape mutants, which usually develop upon individual application of any one of these antibodies. It could further be shown that both antibody types are cross-reactive, i.e., they interact synergistically in that each of them is able to capture the escaped HIV-1 mutants of the other antibody. Combined in a mixture, they are therefore a powerful tool to combat HIV-1 infections and AIDS. It is one of the objects of the present invention to provide for a mixture of at least one antibody of the CL1 type and at least one antibody of the CL2 type.

The present invention also relates to a cell line producing any one of the antibodies described above, and in particular, to the cell lines CL1 through CL6 identified by their accession numbers as described below. Viable samples of the hybridoma cell lines CL1 to CL6 producing the monoclonal antibodies herein described were deposited at the European Collection of Animal Cell Cultures (ECACC) at the Public Health Laboratory Service (PHLS), Centre for Applied Microbiology and Research, Porton Down, Salisbury SP4 0JG, United Kingdom. They are identified by their accession numbers:

CL1—Accession No. 90091704 (deposited on Sep. 17, 1990);

CL2—Accession No. 93091517 (deposited on Sep. 15, 1993);

CL3—Accession No. 95032235 (deposited on Mar. 22, 1995);

CL4—Accession No. 95032236 (deposited on Mar. 22, 1995);

CL5—Accession No. 95032240 (deposited on Mar. 22, 1995); and

CL6—Accession No. 95032241 (deposited on Mar. 22, 1995).

The corresponding monoclonal antibodies produced by these cell lines are hereinafter termed MAb CL1, MAb CL2 through MAb CL6, when used in the abbreviated form.

In a further embodiment of the present invention, peptide fragments are provided which contain at least one of the antigenic determinants of gp41/gp160 and gp120/gp160 as herein described. It is desired that these peptide fragments are of a nature such that they are able to induce an immune response against HIV-1 infection, optionally the production and/or release of HIV-1 neutralizing antibodies after administration to mammals, e.g., to an animal or a human individual.

In another embodiment, these peptide fragments may be linked to a suitable carrier in order to improve the efficacy of antigen presentation to the immune system. Such carriers may be, for instance, organic polymers including proteins, but any other appropriate and physiologically acceptable carrier may also be used, including tetanus toxoid, cholera toxin, keyhole limpet hemocyanin, glutathione S-transferase and all viruses that can be modified by recombinant DNA technology such as, e.g. Rhino-, Polio-, Vaccinia-, or Influenzavirus, etc. It may be advantageous in many cases to have the peptide fragments linked to a modifies, i.e., attenuated and/or recombinant virus such as modified influenza virus or modified hepatitis B virus or to parts of a virus, e.g., to a viral glycoprotein such as, e.g., hemagglutinin of influenza virus or surface antigen of hepatitis B virus, in order to increase the immunological response against HIV-1 viruses and/or infected cells.

It is also an important object of the present invention to provide for the manufacture of a reliable vaccine to protect

people from HIV-1 infection and/or to treat patients with already manifest HIV-1 infections in the course of a therapy. Vaccines based on at least one of the idiotypic antibodies of the CL2 and CL1 groups can be employed for active immunization in the prophylaxis and therapy of higher animals including man. Convincing evidence are provided below for the reduction of the HIV-1 level in the plasma and serum of a seropositive patient in the course of a therapeutic treatment in a preclinical study (cf. Example 8 and FIG. 6). Also, the preventive potency of the idiotypic antibodies of cell line CL1 was demonstrated in an impressive SCID-mouse trial as well as in a chimpanzee experiment. Neither the antibody-treated mice nor the chimpanzees developed HIV-1 infection upon challenge with live HIV-1 virus, while the animals in the untreated control groups became infected.

The use of at least one anti-idiotypic antibody as hereinbefore described for the manufacture of a vaccine for active immunization can help to successfully combat HIV-1 infection. The anti-idiotypic antibodies—as well as the drugs and vaccines derived therefrom—may primarily be used for the preventive treatment of HIV-1 endangered people and are optimally applied prior to coming into contact with HIV-1 virus. Due to their unique paratope characteristics they may also be administered to already infected patients in order to stimulate the immune system to release the corresponding—and possibly even more powerful—HIV-1 neutralizing antibodies. They may be either directly administered to a person or in combination with at least one suitable carrier and/or additive as usual in the art, and/or along with additional drugs such as, for instance, nucleoside analogues (e.g. AZT, ddI), cytokines (e.g. interleukins), HIV-protease inhibitors, antibiotics, etc. The anti-idiotypic antibodies may, however, also serve as “model templates” for the design and construction of, e.g., fusion proteins carrying their respective antigenic determinant(s) (paratopes).

It might be preferable in many cases to combine an individual antibody or a mixture of at least two different antibodies with an immunosuppressant and/or an antibiotic, in order to further improve the benefit of an antibody vaccine manufactured accordingly.

In other cases it might be advantageous to use at least one of the herein specified antigenic peptide fragments of gp41/gp160 and gp120/gp160 to substitute the anti-idiotypic antibodies in the corresponding vaccines and drugs. Therefore the present invention also relates to said peptide fragments and to the use thereof for the manufacture of drugs and/or vaccines applicable in the prophylactic and/or therapeutic treatment of HIV-1 endangered or HIV-1 infected people. The fragments are preferably applied as fusion proteins, wherein they are linked to a suitable carrier which might be a recombinant or attenuated virus or a part of a virus such as, e.g., the hemagglutinin of influenza virus or the surface antigen of hepatitis B virus, or another suitable carrier including other viral surface proteins, e.g., surface proteins of Rhinovirus, Poliovirus, Sindbis virus, Cocksackievirus, etc., for efficient presentation of the antigenic site(s) to the immune system. In some cases, the antigenic fragments might, however, also be purely, i.e., without attachment to a carrier, applied in an analytical or therapeutic program. It is of considerable benefit that the fragments can be used for the prevention and/or treatment of HIV-1 infections in human individuals such as persons belonging to one of the high-risk groups of HIV-1 endangered people including medical and scientific staff dealing with HIV-1 viruses and/or infected individuals.

The idiotypic antibodies referred to herein may further be used for the detection and/or determination of HIV-1 infected cells and/or HIV-1 viruses, either as individual antibodies or as an antibody cocktail. Similarly, one or more of the anti-idiotypic antibodies and/or the above-specified

peptide fragments can successfully be applied to detect and/or determine anti-HIV-1 antibodies binding to the viruses or to HIV-1 infected cells. Both the idiotypic and anti-idiotypic antibodies of the present invention may therefore be prepared and arranged for an analytical testing procedure and/or for a commercially utilizable test kit.

Finally, it is also an object of the present invention to provide a method of treating HIV-1 infected persons in need of such treatment, and to provide for a method of preventing people from becoming HIV-1 infected. Patients with manifest HIV-1 infections may be therapeutically treated with a vaccine comprising at least one of the idiotypic antibodies of the CL2 and the CL1 type, preferably a mixture thereof. However, in some cases it might be preferable to administer at least one of the anti-idiotypic antibodies and/or antigenic peptide fragments in order to induce additional—possibly even more powerful—antibodies to neutralize the viruses and to reduce the HIV-1 levels in the blood of infected patients.

The vaccine based on antibodies and/or antigenic peptide fragments may further comprise suitable, i.e., physiologically acceptable, carriers—preferably for the preparation of injection solutions—and further additives as usually applied in the art (stabilizers, preservatives, etc.), as well as additional drugs. The patients may be administered a dose of approximately 1 to 10 $\mu\text{g/kg}$ body weight, preferably by intravenous injection once a day. For less threatening cases or long-lasting therapies the dose may be lowered to 0.5 to 5 $\mu\text{g/kg}$ body weight per day. The treatment may be repeated in periodic intervals, e.g.; two to three times per day, or in daily or weekly intervals, depending on the status of the infection.

Vaccines according to the present invention may comprise any one of the idiotypic or anti-idiotypic antibodies or any one of the peptide fragments disclosed herein, either alone or in combination with suitable carriers and/or linked to carrier molecules. In some cases, e.g., where HIV-1 infection is acute and/or has already considerably progressed, it might be preferable to apply a mixture of idiotypic antibodies, while in other cases it might be more beneficial to apply a mixture of anti-idiotypic antibodies and/or—preferably carrier-linked—gp160 peptide fragments. It is recommended to apply a dose of 0.5 to 10 $\mu\text{g/kg}$ body weight of antibody or carrier-linked gp160 peptide fragments, administered once to three times a day and possibly repeated in periodic intervals, e.g., weekly, monthly or yearly intervals, depending on the status of HIV-1 infection or the estimated threat of an individual of getting HIV infected.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and a riot to be construed as limiting this invention in any respect.

EXAMPLE 1 (FIG. 1)

Reactivity of GST/HIV-gp120 Fusion Proteins with Antibodies from Cell Line CL2

The binding characteristics of the human monoclonal antibody produced by CL2 (referred to hereinafter as MAB CL2) to the HIV-1 envelope glycoprotein gp120:

Overlapping gp120 fragments were fused to Glutathione S-transferase (GST) and expressed using the insect cell/baculovirus system. Cell lysates of SF9 cells infected with recombinant baculovirus clones expressing different GST-gp120 fragments were first tested for their production level of GST. Lysates of GST-gp120 fusion proteins were then analysed in order to determine the binding affinity of MAB CL2. OD values of MAB CL2 given in the figure correspond to different rgp120 fragments. Microtiter plates were pre-coated with 2 $\mu\text{g/ml}$ glutathione, cell lysates were added and incubated for 1 hour followed by an incubation step of 1

hour with 1 µg/ml MAb CL2 and detection with horseradish peroxidase conjugated anti-human IgG. The optical densities of the cell lysates corresponding to an equal amount of the GST fusion protein are shown.

In FIG. 1, GST-fusion-protein containing fragment 1 corresponds to amino acids 1-95 of processed gp120 of the BH10 isolate of HIV-1, fragment 2 corresponds to amino acids 79-184 of gp120, fragment 3 to amino acids 170-279, fragment 4 to amino acids 264-354, fragment 5 to amino acids 326-400 and fragment 6 to amino acids 384-481. FIG. 1 demonstrates that MAb CL2 binds to two different fragments of gp120, namely to fragment 2 (amino acids 79-184) and to fragment 5 (amino acids 326-400).

EXAMPLE 2 (FIG. 2)

Antibody Binding to Deglycosylated gp160_{HIV MN}

For N-deglycosylation protein samples (500 ng recombinant gp160 of HIV-1 isolate MN) were boiled 10 min. in denaturation buffer (0.5% SDS, 1% β-Mercaptoethanol). Then 1/10 volume each of 10× enzyme reaction buffer and 10% NP-40 (polyglycol ether surfactant; Tergitol®) were added. This reaction mixture was incubated with 2000 U of PNGaseF (Boehringer Mannheim) for 12 hours at 37° C. Polyacrylamide gel electrophoresis was performed on gels in 10-20% Tris/Glycine. After protein blotting, identical membranes were incubated with 5 µg/ml MAb CL2 (panel A), and 5 µg/ml MAb CL1 (panel B) as control.

In FIG. 2 lanes 1 to 3 contain the following:

lanes 1: untreated gp160_{HIV MN};

lanes 2: gp160_{HIV MN} conditioned for PNGaseF treatment without enzyme;

lanes 3: gp160_{HIV MN} PNGaseF treated;

molecular weight markers are indicated in kDa,

It can be seen in FIG. 2 that MAb CL2 does not bind to gp160 after the deglycosylating action of PNGaseF (panel A, lane 3), while MAb CL1 binds to the PNGaseF treated gp160 (panel B, lane 3).

EXAMPLE 3 (FIG. 3)

Reactivity of Recombinant GST/HIVgp120 Fusion Protein with MAb CL2 and anti-GST Antiserum in the Presence and Absence of Tunicamycin (TM)

SF9 insect-cells were infected with either wildtype baculovirus or GST-gp120 expressing recombinant baculovirus. 5 hours after infection, tunicamycin was added to a final concentration of 5 µg/ml. Cells were harvested after 48 hours and lysed. Anti-GST reactivity and MAb CL2 reactivity were tested by ELISA. Baculovirus infected cell-lysates (obtained from 1×10⁷ cells/ml) were transferred to microtiter plates, which were precoated with 2 µg/ml glutathione and incubated for 1.5 hours. GST-fusion protein or gp120 was detected by GST-antiserum (diluted 1:2000) or MAb 2G12 (1.5 µg/ml), respectively, and horseradish peroxidase conjugated anti-mouse/anti-human IgG. The absorbance was determined at 492 nm.

As can be seen in FIG. 3 MAb CL2 does not bind to the gp120 fusion protein in the presence of tunicamycin, whereas anti-GST does, although at a decreased level.

EXAMPLE 4

Neutralization Capacity of MAb CL2 for Primary HIV-1 Isolates

A PBMC (=peripheral blood mononuclear cells) based neutralization assay was performed as described by Purtscher et al. (M. Purtscher et al., 1994, Aids Research and Human Retroviruses. 10/12: 1651-1658, Mary Ann Liebert, Inc., Publ., 1994) by pre-incubating virus with serial antibody dilutions for 1 h at 37° C. and subsequent infection of fresh PHA (=phytohemagglutinin) stimulated PBMC prepared from HIV-negative donor buffy-coat cells. Neutral-

ization capacity was estimated after 7 to 12 days by comparing the amounts of p24 antigen produced by the cells in the presence or in the absence of antibody.

TABLE 1

Summary of neutralization capacity of MAb CL2 in neutralization assays using PBMC		
primary isolate	subtype clade	neutralizing capacity
92RW009	A	+++
92RW021	A	+++
92UG037	A	+++
92TH014	B	+++
92BR030	B	+++
N70-2	B	+
DJ259	C	+
WYG	unknown	+++
WRF	unknown	+++
WRB	unknown	+++
WSC	unknown	+
WHM	unknown	+

Key:

+++ 90% neutralization at a conc. of <1 µg/ml

++ 90% neutralization at a conc. between 1 to 50 µg/ml

+ 50% neutralization at a conc. below 50 µg/ml

EXAMPLE 5 (FIG. 4)

Syncytia Inhibition Assay/Anti-idiotype Blocking

An anti-idiotype (Ab2) blocking assay was performed to determine whether the anti-idiotypic antibodies Ab2 block the neutralization capacity of MAb CL2 by binding to the neutralizing paratope of MAb CL2. The syncytia inhibition concentrations (EC₅₀) of MAbs CL1 and CL2 in the absence of anti-idiotypic antibodies were 2.0 and 8.8 µg/ml, respectively (the HIV-1 isolate RF was used). The addition of anti-idiotypic antibodies to MAb CL2 revealed that the antibodies M1A3 and M4C12 did not alter the neutralizing capacity, but when M1G1 (=anti-idiotypic antibody produced by CL6) was incubated with MAb CL2 a significant impairment could be observed (Table 2). The syncytia inhibiting capacity of MAb CL1, which is directed against gp41, should not be affected by the anti-idiotypic antibodies tested. No syncytia inhibition was observed with anti-idiotypic antibodies alone at a concentration of 100 µg/ml as well as with MAb 3D6, which was used as a non-neutralizing control.

TABLE 2

EC ₅₀ of MAb CL2 and MAb CL1 in the presence of M1G1, M1A3 or M4C12				
MAb	alone	anti-idiotype added (µg/ml)		
		+ M1G1 (= MAb CL6) 100	+ M1A3 (= MAb CL5) 100	+ M4C12 100
MAb CL2	2.02	5.26	2.63	0.66
MAb CL1	8.83	7.43	8.83	6.25

Anti-idiotypic antibodies were diluted to 200 µg/ml and MAbs CL2 and CL1 (as control antibody) were diluted to 10 µg/ml in RPMI 1640 medium 50 µl of serial two-fold dilutions of MAbs CL2 and CL1 were prepared starting at 100 µg/ml in four replicates. 50 µl of anti-idiotypic antibody (200 µg/ml) were added to each well and pre-incubated for 1 h at 37° C. in the incubator. As virus inoculum the HIV-1 isolate RF was diluted to approximately 10²-10³ TCID₅₀/ml and 50 µl of the virus suspension were added to each well. After an incubation of 1 h at 37° C., 50 µl of AA-2 cell

suspension (10^4 cells per ml; see CHAFFEE et al, 1988, J.Exp.Med. 168:605-621) were added to each well. The cells were then cultivated during 5 days at 37° C. and 5% CO₂, followed by microscopical evaluation of syncytia formation. Occurrence of at least one syncytium per well was recorded as an indication of HIV-1 infection. The 50% effective concentration (EC₅₀) was calculated by the method of Reed and Muench (Reed, L. J. and Muench, H. 1938. A simple method of estimating fifty percent endpoints. Am.J.Hyg.27:493-497).

All wells of one dilution step were then pooled and p24 was determined quantitatively in a p24 antigen ELISA. The measured p24 values were plotted against the MAb concentration. The results thereof can be seen in FIG. 4 which displays the production of antigen p24 in cultures containing MAb CL2 (graph A) and MAb CL1 (graph B) with different amounts of anti-idiotypic antibodies. Contrary to M1A3 (=MAb CL5) and 4C12, M1G1 (=MAb CL6) inhibits the neutralization activity of MAb CL2 suggesting that M1G1 at least partly mimics the paratope (of HIV-1 gp120) corresponding to the epitope of MAb CL2. The control MAb CL1 is not inhibited by any of these antibodies. These results indicate that monoclonal antibody M1G1 is an anti-idiotypic antibody to MAb CL2.

EXAMPLE 6 (FIG.5)

Reactivity of the Anti-idiotypic Antibodies with Different Anti-gp160 Antibodies

A panel of human anti-gp160 antibodies and pooled serum of HIV-1 positive individuals (HIVIG) were incubated on gp160-coated microtiter plates in the presence of M1G1 (=MAb CL6) and M1A3 (=MAb CL5), to prove the reactivity of the anti-idiotypes. FIG. 5, graphs A and B, show the MAb CL2 specific binding of M1G1 (graph A) and M1A3 (graph B), respectively. Both anti-idiotypic antibodies were only reactive with MAb CL2 and its recombinant double (MAb CL3) but not with other tested human antibodies (MAb CL1 and 5F3 and HIVIG are representative examples of human anti-HIV-1 antibodies).

FIG. 5, graphs A and B: 96-microtiter plates were coated with 2 µg/ml gp160 (Immuno AG, Vienna). Starting dilution of the human monoclonal antibody samples began at a concentration of about 200 ng/ml and HIVIG was prediluted 1:100. Eight dilutions of the human antibodies were performed in 2" steps. M1G1 and M1A3 were used at a concentration of 1 µg/ml. The human and murine antibody dilutions were transferred to the test plate and simultaneously incubated for 1 h. Then peroxidase-conjugated goat anti-mouse IgG was applied to the plate. After 1 h of incubation staining solution was added to each well; the absorbances were read at 492 nm against 620 nm.

EXAMPLE 7

Immune Selection Experiments with HIV-1 Molecular Clone cl82.

Immune selection experiments with HIV-1 molecular clone cl82 in the presence of either MAb CL1 or MAb CL2 resulted in the generation of escape mutants at antibody concentrations lower than 6.3 µg/ml and 25 µg/ml, respectively. When both MAbs were present at the same time in the reaction mixtures virus variants evolved were only resistant to either MAb CL1 or MAb CL2. These results (Table 3) suggest that the virus cannot accumulate sufficient mutations to render itself resistant to both antibodies. Therefore, the emergence of HIV-1 escape mutants during treatment of HIV-1 infected individuals could be overcome by using a mixture of MAb CL1 and MAb CL2.

TABLE 3

	MAb	Selection conditions		
		µg/ml	virus emerged	EC ₅₀ > 50 µg/ml
5	MAbCL1	25	no	
		6.3	no	
		1.6	yes	+
		0.8	yes	+
10	MAb CL2	25	no	
		6.3	yes	+
		1.6	yes	+
		0.8	ND	
15	MAb CL1 + CL2 (Mixture: 50/50)	25	no	
		6.3	no	
		1.6	no	
		0.8	1)	-
20		0.4	2)	-

ND, not done

1) neutralization resistant to MAb CL2; Mixture and MAb CL1 still neutralizing;

2) neutralization resistant to MAb CL1; Mixture and MAb CL2 still neutralizing.

EXAMPLE 8 (FIG. 6)

Course of p24 Production in Cultures with Serum Samples from an HIV-1 Infected Individual Before and after Treatment with 3 Doses of MAb CL1.

Serum was incubated with PHA-stimulated PBMC from healthy, HIV-negative blood donors. Twice a week, culture supernatant was changed 1:2 by removing half of the supernatant and substituting therefor an equal volume of fresh media. Once per week fresh PHA-stimulated PBMC were added to the culture. The culture was monitored for 5 weeks.

FIG. 6 shows the increase in syncytia formation of cultured serum samples taken from the patient before the administration of MAb CL1 (triangles) and the impressive neutralization of the patient's HIV-1 infection upon administration of MAb CL1, as displayed by the horizontal line at the zero level of p24 production.

EXAMPLE 9

In Vivo Prevention of HIV-1 Infection in a Chimpanzee

4 chimpanzees have been selected for testing the in vivo neutralization in a collaboration with the Merck-research center in West Point, Pa. 19486, USA.

Prior to the in vivo test, CD4 positive primary T cells (PBMC's) were isolated from each chimpanzee to test the permissiveness of in vitro infection with the primary HIV-1 isolate, lade B. For all in vitro tests, conventional procedures as described in M. Purtscher et al., Aids Research and Human Retroviruses, Vol. 10, Nr. 12, 1994, Mary Ann Liebert, Inc., Publ., have been used. The CD4 PBMC of all four chimpanzees were permissive to viral propagation in vitro. This was to prove that an in vivo infection should be successful.

Two of the chimpanzees were infused with the monoclonal antibody CL1 (PHLS Deposit No. 90091704). For the intravenous infusion of the antibodies 300 ml of solution containing approx. 1 mg/per milliliter stabilized in 1% human serum albumin, at pH 7 were applied per animal. Two other animals were only treated with human serum albumin.

All four chimpanzees were challenged one day after treatment with the primary HIV-1 isolate by intravenous injection of 3 chimpanzee infective doses of the virus. All four animals were routinely tested for HIV-1 infection for a period of four months.

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Result: The two chimpanzees treated with antibody CL1 showed no signs of HIV-1 infection; they have been protected from infection. Both control animals, i.e., those treated only with human serum albumin, became HIV-1 positive.

EXAMPLE 10

In Vivo Prevention of HIV-1 Infection in a SCID-mouse Trial

Another experiment to demonstrate the in-vivo neutralization ability of MAb CL1 has been carried out in collaboration with Transgene, Strasbourg, France.

The genes encoding the heavy and light chains of MAb CL1 have been supplied to Transgene to genetically manipulate mouse fibroblasts (3T3) using standard genetic engineering techniques. The transformed mouse fibroblasts producing MAb CL1 were propagated in vitro on GOREDEX® fibres to form cell pellets. The cell pellets were then applied under the skin of SCID mice to form organelles within these mice so as to release the MAb CL1 into the blood stream.

The SCID-mice were reconstituted using conventional procedures with the human white blood cell system in order to give an animal model suitable for infection by HIV-1.

Those SCID-mice having a level of MAb CL1 higher than 2 micrograms of antibody per ml serum were protected against a challenge with HIV-1 IIIB, whereas those having a lower level of antibody per ml in the serum showed a significant delay of infection. SCID-mice treated otherwise in an analogous way and having no MAb CL1 in their serum were all infected.

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EXAMPLE 11

Detection of HIV-infection by Means of HIV-1 Peptide Fragments and/or Antiidiotypic Antibodies

Peptide fragments according to the present invention containing at least one of the antigenic determinants of gp41/gp160 and gp120/gp160 as herein described and/or antiidiotypic antibodies recognizing and binding to the epitope of MAb CL1 or MAb CL2 are coated onto microtiter plates by known procedures. Then, sera or plasma of HIV-1 infected patients are added to the precoated wells, whereupon anti-HIV-1 antibodies captured by the HIV-1 specific peptide fragments and/or by said antiidiotypic antibodies are detected by an anti-human IgG specific antibody conjugate (e.g. IgG-horseradish peroxidase) in an ELISA. The presence of antibodies that bind to HIV-1 specific peptide fragments indicate an infection with HIV.

EXAMPLE 12

Detection of HIV-infection by Means of Anti-HIV Antibodies

PBMC from HIV-1 endangered or infected patients are isolated by a Ficoll® density gradient centrifugation. Cells are then incubated with at least one of the HIV-1 neutralizing antibodies MAb CL1, MAb CL2, MAb CL3 and MAb CL4 and/or with functionally equivalent antibodies obtained upon active immunization of an animal or human individual with an antiidiotypic antibody such as MAb CL6 (M1G1) or MAb CL5 (M1A3). Incubation is carried out at standard conditions e.g., at room temperature or at 37° C. for about one hour, or at 4° C. overnight. Bound antibody, confirming an HIV infection, is detected by a fluorochrome conjugated anti-human IgG antibody and analyzed in a fluorescence activated cell scanner (FACS).

SEQUENCE LISTING

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<212> TYPE: DNA

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gcaacaatga gtccagagac ttcagacctg gaggaaggaga tatgaggagc aattggagaa	7020
gtgaattata taatatataa gtagtaaaaa ttgaaccatt aggagtagca cccaccaagg	7080
caaagagaag agtggtcgag agagaaaaaa gagcagtggt aataggagct ttgttccctg	7140
ggttcttggg agcagcagga agcactatgg gcgcagcgtc aatgacgctg acggtacagg	7200
ccagacaatt attgtctggt atagtgcagc agcagaacaa ttgtctgagg gctattgagg	7260
cgcaacagca tctgttgcaa ctcacagtct ggggcaccaa gcagctccag gcaagaatcc	7320
tggctgtgga aagataccta aaggatcaac agctcctggg gatttgggtg tgctctgga	7380
aactcatttg caccactgct gtgccttgga atgctagtgt gagtaataaa tctctggaac	7440
agatttgga taacatgacc tggatggagt gggacagaga aattaacaat tacacaagct	7500
taatacactc cttaattgaa gaatcgcaaa accagcaaga aaagaatgaa caagaattat	7560
tggaaattaga taatgggca agtttgtgga attggtttaa catacaaat tggctgtggt	7620
atataaaatt attcataatg atagtaggag gcttggtagg tttaagaata gtttttgctg	7680
tactttctgt agtgaataga gttaggcagg gatattcacc attatcgttt cagacccacc	7740
tcccaatccc gaggggaccc gacaggcccg aaggaaataga agaagaaggt ggagagagag	7800
acagagacag atccattcga ttagtgaacg gatccttagc acttatctgg gacgatctgc	7860
ggagcctgtg cctcttcagc taccaccgct tgagagactt actcttgatt gtaacgagga	7920
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agtattggag tcaggagcta aagaatagt ctgttagctt gctcaatgcc acagctatag 8040
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gccacatacc tagaagaata agacagggct tggaaaggat ttgtctataa gatgggtggc 8160
aagtggctca aagtagtgtt ggttggatgg cctgctgtaa gggaaagaat gagacgagct 8220
gagccagcag cagatggggt gggagcagca tctcgagacc tagaaaaaca tggagcaatc 8280
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<210> SEQ ID NO 2

<211> LENGTH: 512

<212> TYPE: PRT

<213> ORGANISM: Human immunodeficiency virus type 1

<400> SEQUENCE: 2

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Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp
1           5           10           15
Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys
20           25           30
His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro
35           40           45
Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu
50           55           60
Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn
65           70           75           80
Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Glu Ile Lys Asp
85           90           95
Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys
100          105          110
Lys Lys Ala Gln Gln Ala Ala Asp Thr Gly His Ser Ser Gln Val
115          120          125
Ser Gln Asn Tyr Pro Ile Val Gln Asn Ile Gln Gly Gln Met Val His
130          135          140
Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu
145          150          155          160
Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser
165          170          175
Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly
180          185          190
Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu
195          200          205

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Ala Ala Glu Trp Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala
 210 215 220
 Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr
 225 230 235 240
 Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile
 245 250 255
 Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys
 260 265 270
 Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly
 275 280 285
 Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu
 290 295 300
 Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr
 305 310 315 320
 Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala
 325 330 335
 Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly
 340 345 350
 Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser
 355 360 365
 Gln Val Thr Asn Thr Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg
 370 375 380
 Asn Gln Arg Lys Met Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His
 385 390 395 400
 Thr Ala Arg Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys
 405 410 415
 Gly Lys Glu Gly His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn
 420 425 430
 Phe Leu Gly Lys Ile Trp Pro Ser Tyr Lys Gly Arg Pro Gly Asn Phe
 435 440 445
 Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Phe Leu Gln Ser Arg
 450 455 460
 Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Ser Gly Val Glu
 465 470 475 480
 Thr Thr Thr Pro Pro Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr
 485 490 495
 Pro Leu Thr Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln
 500 505 510

<210> SEQ ID NO 3

<211> LENGTH: 1015

<212> TYPE: PRT

<213> ORGANISM: Human immunodeficiency virus type 1

<400> SEQUENCE: 3

Phe Phe Arg Glu Asp Leu Ala Phe Leu Gln Gly Lys Ala Arg Glu Phe
 1 5 10 15
 Ser Ser Glu Gln Thr Arg Ala Asn Ser Pro Thr Ile Ser Ser Glu Gln
 20 25 30
 Thr Arg Ala Asn Ser Pro Thr Arg Arg Glu Leu Gln Val Trp Gly Arg
 35 40 45
 Asp Asn Asn Ser Pro Ser Glu Ala Gly Ala Asp Arg Gln Gly Thr Val
 50 55 60
 Ser Phe Asn Phe Pro Gln Ile Thr Leu Trp Gln Arg Pro Leu Val Thr
 65 70 75 80

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Ile Lys Ile Gly Gly Gln Leu Lys Glu Ala Leu Leu Asp Thr Gly Ala
 85 90 95
 Asp Asp Thr Val Leu Glu Glu Met Ser Leu Pro Gly Arg Trp Lys Pro
 100 105 110
 Lys Met Ile Gly Gly Ile Gly Gly Phe-Ile Lys Val Arg Gln Tyr Asp
 115 120 125
 Gln Ile Leu Ile Glu Ile Cys Gly His Lys Ala Ile Gly Thr Val Leu
 130 135 140
 Val Gly Pro Thr Pro Val Asn Ile Ile Gly Arg Asn Leu Leu Thr Gln
 145 150 155 160
 Ile Gly Cys Thr Leu Asn Phe Pro Ile Ser Pro Ile Glu Thr Val Pro
 165 170 175
 Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro
 180 185 190
 Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met
 195 200 205
 Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn
 210 215 220
 Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys
 225 230 235 240
 Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu
 245 250 255
 Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser
 260 265 270
 Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp
 275 280 285
 Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn
 290 295 300
 Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp
 305 310 315 320
 Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu
 325 330 335
 Pro Phe Lys Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp
 340 345 350
 Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys
 355 360 365
 Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro
 370 375 380
 Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu
 385 390 395 400
 Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys
 405 410 415
 Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn
 420 425 430
 Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys
 435 440 445
 Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu
 450 455 460
 Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro
 465 470 475 480
 Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile
 485 490 495

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Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro	500	505	510
Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His	515	520	525
Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr	530	535	540
Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile	545	550	555
Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr	565	570	575
Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu	580	585	590
Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr	595	600	605
Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr	610	615	620
Val Thr Asn Lys Gly Arg Gln Lys Val Val Pro Leu Thr Asn Thr Thr	625	630	635
Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser	645	650	655
Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile	660	665	670
Ile Gln Ala Gln Pro Asp Lys Ser Glu Ser Glu Leu Val Asn Gln Ile	675	680	685
Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro	690	695	700
Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser	705	710	715
Ala Gly Ile Arg Lys Ile Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln	725	730	735
Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp	740	745	750
Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp	755	760	765
Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser	770	775	780
Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile	785	790	795
Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile	805	810	815
Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala	820	825	830
Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe	835	840	845
Thr Ser Ala Thr Val Lys Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln	850	855	860
Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser	865	870	875
Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala	885	890	895
Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe	900	905	910

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Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val
 915 920 925
 Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile
 930 935 940
 Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro
 945 950 955 960
 Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val
 965 970 975
 Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala
 980 985 990
 Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val
 995 1000 1005
 Ala Ser Arg Gln Asp Glu Asp
 1010 1015

<210> SEQ ID NO 4
 <211> LENGTH: 192
 <212> TYPE: PRT
 <213> ORGANISM: Human immunodeficiency virus type 1

<400> SEQUENCE: 4

Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
 1 5 10 15
 Arg Ile Arg Thr Trp Lys Ser Leu Val Lys His His Met Tyr Val Ser
 20 25 30
 Gly Lys Ala Arg Gly Trp Phe Tyr Arg His His Tyr Glu Ser Pro His
 35 40 45
 Pro Arg Ile Ser Ser Glu Val His Ile Pro Leu Gly Asp Ala Arg Leu
 50 55 60
 Val Ile Thr Thr Tyr Trp Gly Leu His Thr Gly Glu Arg Asp Trp His
 65 70 75 80
 Leu Gly Gln Gly Val Ser Ile Glu Trp Arg Lys Lys Arg Tyr Ser Thr
 85 90 95
 Gln Val Asp Pro Glu Leu Ala Asp Gln Leu Ile His Leu Tyr Tyr Phe
 100 105 110
 Asp Cys Phe Ser Asp Ser Ala Ile Arg Lys Ala Leu Leu Gly His Ile
 115 120 125
 Val Ser Pro Arg Cys Glu Tyr Gln Ala Gly His Asn Lys Val Gly Ser
 130 135 140
 Leu Gln Tyr Leu Ala Leu Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
 145 150 155 160
 Pro Pro Leu Pro Ser Val Thr Lys Leu Thr Glu Asp Arg Trp Asn Lys
 165 170 175
 Pro Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
 180 185 190

<210> SEQ ID NO 5
 <211> LENGTH: 78
 <212> TYPE: PRT
 <213> ORGANISM: Human immunodeficiency virus type 1

<400> SEQUENCE: 5

Met Glu Gln Ala Pro Glu Asp Gln Gly Pro Gln Arg Glu Pro His Asn
 1 5 10 15
 Glu Trp Thr Leu Glu Leu Leu Glu Glu Leu Lys Asn Glu Ala Val Arg
 20 25 30

-continued

His Phe Pro Arg Ile Trp Leu His Gly Leu Gly Gln His Ile Tyr Glu
 35 40 45
 Thr Tyr Gly Asp Thr Trp Ala Gly Val Glu Ala Ile Ile Arg Ile Leu
 50 55 60
 Gln Gln Leu Leu Phe Ile His Phe Gln Asn Trp Val Ser Thr
 65 70 75

<210> SEQ ID NO 6
 <211> LENGTH: 86
 <212> TYPE: PRT
 <213> ORGANISM: Human immunodeficiency virus type 1

<400> SEQUENCE: 6

Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
 1 5 10 15
 Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Phe
 20 25 30
 His Cys Gln Val Cys Phe Ile Thr Lys Ala Leu Gly Ile Ser Tyr Gly
 35 40 45
 Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln Gly Ser Gln Thr
 50 55 60
 His Gln Val Ser Leu Ser Lys Gln Pro Thr Ser Gln Ser Arg Gly Asp
 65 70 75 80
 Pro Thr Gly Pro Lys Glu
 85

<210> SEQ ID NO 7
 <211> LENGTH: 116
 <212> TYPE: PRT
 <213> ORGANISM: Human immunodeficiency virus type 1

<400> SEQUENCE: 7

Met Ala Gly Arg Ser Gly Asp Ser Asp Glu Asp Leu Leu Lys Ala Val
 1 5 10 15
 Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn Pro Pro Pro Asn Pro Glu
 20 25 30
 Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg Arg Trp Arg Glu Arg
 35 40 45
 Gln Arg Gln Ile His Ser Ile Ser Glu Arg Ile Leu Ser Thr Tyr Leu
 50 55 60
 Gly Arg Ser Ala Glu Pro Val Pro Leu Gln Leu Pro Pro Leu Glu Arg
 65 70 75 80
 Leu Thr Leu Asp Cys Asn Glu Asp Cys Gly Thr Ser Gly Thr Gln Gly
 85 90 95
 Val Gly Ser Pro Gln Ile Leu Val Glu Ser Pro Thr Val Leu Glu Ser
 100 105 110
 Gly Ala Lys Glu
 115

<210> SEQ ID NO 8
 <211> LENGTH: 81
 <212> TYPE: PRT
 <213> ORGANISM: Human immunodeficiency virus type 1

<400> SEQUENCE: 8

Met Gln Pro Ile Gln Ile Ala Ile Val Ala Leu Val Val Ala Ile Ile
 1 5 10 15

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Ile Ala Ile Val Val Trp Ser Ile Val Ile Ile Glu Tyr Arg Lys Ile
 20 25 30
 Leu Arg Gln Arg Lys Ile Asp Arg Leu Ile Asp Arg Leu Ile Glu Arg
 35 40 45
 Ala Glu Asp Ser Gly Asn Glu Ser Glu Gly Glu Ile Ser Ala Leu Val
 50 55 60
 Glu Met Gly Val Glu Met Gly His His Ala Pro Trp Asp Val Asp Asp
 65 70 75 80
 Leu

<210> SEQ ID NO 9
 <211> LENGTH: 856
 <212> TYPE: PRT
 <213> ORGANISM: Human immunodeficiency virus type 1
 <220> FEATURE:
 <221> NAME/KEY: PEPTIDE
 <222> LOCATION: (79)..(184)
 <221> NAME/KEY: PEPTIDE
 <222> LOCATION: (326)..(400)

<400> SEQUENCE: 9

Met Arg Val Lys Glu Lys Tyr Gln His Leu Trp Arg Trp Gly Trp Arg
 1 5 10 15
 Trp Gly Thr Met Leu Leu Gly Met Leu Met Ile Cys Ser Ala Thr Glu
 20 25 30
 Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala
 35 40 45
 Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu
 50 55 60
 Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn
 65 70 75 80
 Pro Gln Glu Val Val Leu Val Asn Val Thr Glu Asn Phe Asn Met Trp
 85 90 95
 Lys Asn Asp Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp
 100 105 110
 Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Ser
 115 120 125
 Leu Lys Cys Thr Asp Leu Lys Asn Asp Thr Asn Thr Asn Ser Ser Ser
 130 135 140
 Gly Arg Met Ile Met Glu Lys Gly Glu Ile Lys Asn Cys Ser Phe Asn
 145 150 155 160
 Ile Ser Thr Ser Ile Arg Gly Lys Val Gln Lys Glu Tyr Ala Phe Phe
 165 170 175
 Tyr Lys Leu Asp Ile Ile Pro Ile Asp Asn Asp Thr Thr Ser Tyr Thr
 180 185 190
 Leu Thr Ser Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro Lys Val
 195 200 205
 Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Phe Ala
 210 215 220
 Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr
 225 230 235 240
 Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val Ser
 245 250 255
 Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val Ile
 260 265 270

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Arg Ser Ala Asn Phe Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu	275	280	285
Asn Gln Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg	290	295	300
Lys Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile	305	310	315 320
Gly Lys Ile Gly Asn Met Arg Gln Ala His Cys Asn Ile Ser Arg Ala	325	330	335
Lys Trp Asn Asn Thr Leu Lys Gln Ile Asp Ser Lys Leu Arg Glu Gln	340	345	350
Phe Gly Asn Asn Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp	355	360	365
Pro Glu Ile Val Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr	370	375	380
Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp	385	390	395 400
Ser Thr Lys Gly Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu	405	410	415
Pro Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys	420	425	430
Ala Met Tyr Ala Pro Pro Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn	435	440	445
Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Asn Asn Glu	450	455	460
Ser Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg	465	470	475 480
Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val	485	490	495
Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala	500	505	510
Val Gly Ile Gly Ala Leu Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser	515	520	525
Thr Met Gly Ala Ala Ser Met Thr Leu Thr Val Gln Ala Arg Gln Leu	530	535	540
Leu Ser Gly Ile Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu	545	550	555 560
Ala Gln Gln His Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu	565	570	575
Gln Ala Arg Ile Leu Ala Val Glu Arg Tyr Leu Lys Asp Gln Gln Leu	580	585	590
Leu Gly Ile Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val	595	600	605
Pro Trp Asn Ala Ser Trp Ser Asn Lys Ser Leu Glu Gln Ile Trp Asn	610	615	620
Asn Met Thr Trp Met Glu Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser	625	630	635 640
Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn	645	650	655
Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp	660	665	670
Phe Asn Ile Thr Asn Trp Leu Trp Tyr Ile Lys Leu Phe Ile Met Ile	675	680	685

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Val Gly Gly Leu Val Gly Leu Arg Ile Val Phe Ala Val Leu Ser Val
690 695 700

Val Asn Arg Val Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr His
705 710 715 720

Leu Pro Ile Pro Arg Gly Pro Asp Arg Pro Glu Gly Ile Glu Glu Glu
725 730 735

Gly Gly Glu Arg Asp Arg Asp Arg Ser Ile Arg Leu Val Asn Gly Ser
740 745 750

Leu Ala Leu Ile Trp Asp Asp Leu Arg Ser Leu Cys Leu Phe Ser Tyr
755 760 765

His Arg Leu Arg Asp Leu Leu Leu Ile Val Thr Arg Ile Val Glu Leu
770 775 780

Leu Gly Arg Arg Gly Trp Glu Ala Leu Lys Tyr Trp Trp Asn Leu Leu
785 790 795 800

Gln Tyr Trp Ser Gln Glu Leu Lys Asn Ser Ala Val Ser Leu Leu Asn
805 810 815

Ala Thr Ala Ile Ala Val Ala Glu Gly Thr Asp Arg Val Ile Glu Val
820 825 830

Val Gln Gly Ala Tyr Arg Ala Ile Arg His Ile Pro Arg Arg Ile Arg
835 840 845

Gln Gly Leu Glu Arg Ile Leu Leu
850 855

<210> SEQ ID NO 10
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: Human immunodeficiency virus type 1

<400> SEQUENCE: 10

Met Gly Gly Lys Trp Ser Lys Ser Ser Val Val Gly Trp Pro Ala Val
1 5 10 15

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Gly Val Gly Ala
20 25 30

Ala Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr
35 40 45

Ala Ala Asn Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Glu Glu
50 55 60

Glu Val Gly Phe Pro Val Thr Pro Gln Val Pro Leu Arg Pro Met Thr
65 70 75 80

Tyr Lys Ala Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly
85 90 95

Leu Glu Gly Leu Ile His Ser Gln Arg Arg Gln Asp Ile Leu Asp Leu
100 105 110

Trp Ile Tyr His Thr Gln Gly Tyr Phe Pro Asp
115 120

<210> SEQ ID NO 11
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Human immunodeficiency virus type 1

<400> SEQUENCE: 11

Glu Leu Asp Lys Trp Ala
1 5

We claim:

1. A peptide fragment which consists of one or both amino acid sequences that correspond to amino acid positions 79 to 184 or 326 to 400 (SEQ ID NO:9) of processed gp120 of HIV-1 isolate BH10 (GenBank accession M15654 (SEQ ID NOS:1-10); numbering described in the Swissprot database entry ENV\$HIV10).

2. The peptide fragment according to claim 1 in combination with a pharmaceutically acceptable carrier.

3. The peptide fragment according to claim 1, linked to a carrier.

4. The peptide fragment according to claim 3, wherein said carrier is a virus or part of a virus.

5. The peptide fragment according to claim 4, wherein said part of a virus is selected from the group consisting of hemagglutinin of influenza virus, surface antigen of hepatitis B virus, surface protein of rhinovirus, surface protein of poliovirus, surface protein of Sindbis virus, and surface protein of coxsackie virus.

6. A pharmaceutical composition comprising at least one peptide fragment as defined in claim 1 or claim 3.

7. A pharmaceutical composition according to claim 6, which comprises said at least one peptide fragment in an amount suitable for administration of 0.5 to 10 $\mu\text{g/kg}$ of body weight.

8. A peptide fragment comprising one or both amino acid sequences that correspond to amino acid positions 79 to 184 or 326 to 400 (SEQ ID NO:9) of processed gp120 of HIV-1 isolate BH10 (GenBank accession M15654 (SEQ ID NOS:1-10); numbering as described in the Swissprot database entry ENV\$HIV10), wherein the peptide fragment in its glycosylated stage binds an HIV-1 neutralizing antibody produced by cell line CL2 (ECACC Accession No. 93091517) or cell line CL3 (ECACC Accession No. 95032235).

* * * * *